Biochemistry

EXPRESSION OF RECOMBINANT WILD TYPE AND MUTANT HUMAN PLASMINOGEN

Thushanthi S. Perera[†], Mariana Figuera[‡], Victoria A. Ploplis[‡], Francis J. Castellino[‡]*

[†] Department of Natural Science, Dominican University, River Forest, Illinois 60305
[‡] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

The human body responds to vascular damage by forming an insoluble fibrin clot; i.e., a local seal that prevents excessive blood loss. After repairing the vascular damage, the body dissolves the clot in order to restore vascular structure. The clot is degraded by plasmin, a potent serine protease, in a process called fibrinolysis. Human plasmin (HPm) forms when its inactive precursor, plasminogen (HPg), is activated by any plasminogen activator.

Since plasminogen is the primary precursor to biologically significant plasmin, individual plasminogen samples form six gene sources were isolated. The wild-type (wt) gene and five mutated (D646E, R561A, D139A, D219A, and D413A) genes for human plasminogen were transfected into *Drosophila melanogaster* with a pMT/BiP/V5-His expression vector. After the insertion of these genes, the S2 cells were cultured in a medium consisting of Schneider's *Drosophila* medium, fetal bovine serum (FBS), and penicillin-streptomycin. When the culture reached a cell density of six million cells per milliliter, copper sulfate ($CuSO_4$) was added to release plasminogen from the S2 cells. The released plasminogen was isolated and purified with affinity chromatography.

The identity and purity of the purified plasminogen was verified by gel electrophoresis and Western blotting. The BCA protein assay then determined the concentration of the purified plasminogen in buffer.